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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/057,505	01/25/2002	Roger Y. Tsien	02307E-151530US	7832

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EXAMINER	
ROBINSON, HOPE A	

ART UNIT	PAPER NUMBER
1652	

MAIL DATE	DELIVERY MODE
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/057,505	TSIEN ET AL.	
	Examiner	Art Unit	
	Hope A. Robinson	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *05 December 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *79-84 and 91-99* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *79-84 and 91-99* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *25 January 2002* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Application Status

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on December 5, 2007 has been entered. It is noted that applicant's filed an IDS on 12/6/07, however, the 1449 was not available for viewing at the time of this office action. Submission of the review will occur under separate cover.

Claim Disposition

2 . Claims 79-84 and 91-99 are pending and are under examination.

New-Claim Rejections - 35 USC ∋ 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

3. Claims 79-84 and 91-99 are rejected under 35 USC112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time

the application was filed, had possession of the claimed invention. The claims recite added material, which is not supported by the original disclosure. Claims 79, 80, 81 and the dependent claims hereto recite "oxidized and cyclized to form a fluorophore" and the phrase "oxidized and cyclized" do not find support in the instant specification. In addition the claims recite "consists of between 5 amino acids and 50 amino acids" which is not supported by the specification which instead discloses at paragraph [0006], "is between 5 and 50 amino acids". Therefore, the specification lacks adequate written description.

Maintained-Claim Rejections - 35 USC ∋ 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

4. Claims 79-81, 85-94, 97 and 99 remain rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The claims are directed to a tandem fluorescent protein construct, comprising a donor fluorescent protein moiety, an acceptor fluorescent protein moiety and a linker moiety that couples the donor and acceptor moieties and wherein the donor and

acceptor moieties exhibit FRET when the donor moiety is excited by radiation, characterized in that the linker moiety comprises a protease cleavage recognition site, wherein cleavage of the linker by a protease results in a change in FRET between the donor and acceptor moieties. The claims are also directed to donor and acceptor moieties comprising an amino acid sequence that is 85% identical' to SEQ ID NO:2 and SEQ ID NO:2 comprising several amino acid substitutions that are combined. The linker is also variable, which can comprise between 5 and 50 amino acids (see for example claim 85 or 12 to 40 (see claim 99). Essentially the claims encompass any structure as long as said structure comprises the above mutation. Thus, the claims encompasses other mutations not defined which could result in a structure that would not produce FRET. There is no indication that run of amino acids considered to be the linker moiety is contiguous residues, which could dramatically affect the donor and acceptor. The claims encompass a genus of proteins not described or defined. Further, the specification fails to provide any additional representative species of the claimed genus to show that applicant was in possession of the claimed genus.

A representative number of species means that the species, which are adequately described are representative of the entire genus. The written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice, disclosure of drawings, or by disclosure of relevant identifying characteristics, for example, structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of

such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus. Therefore, for all these reasons the specification lacks adequate written description, and one of skill in the art cannot reasonably conclude that the applicant had possession of the claimed invention at the time the instant application was filed.

5. Claim 79-81, 85-94, 97 and, 99 remain rejected under 35 U.S.C. 112, first paragraph, because

the specification, while being enabling for a donor fluorescent protein moiety and an acceptor fluorescent moiety contained in SEQ ID NO:2 with specific mutations to SEQ ID NO:2 at the positions listed in for example claim 79 and the disclosure in U.S. Patent No. 5,981,200, (for example, wherein the linker is a peptide moiety that does not emit light to excite the donor fluorescent protein moiety), does not reasonably provide enablement for mutations to the donor and acceptor moieties that are "85% identical to SEQ ID NO:2" that may not produce FRET or similar variability in the linker moiety. In addition, the claims read on any linker and the specification is not enabled for any linker as the linker moiety may refer to a single amino acid or a group or any linker with a protease recognition site for any protease. While the specification is enabled for linkers that are not fluorescent, is not enabled for linkers that are fluorescent. Further, the specification while enabled for linkers about 5-50 amino acids (see page 2 of the specification) is not enabled for linkers with the lengths encompassed in the breath of the claims. For example claim 87 recites "comprises between 5 and 50 amino acids,

which means that the open language "comprises" extends the length in the N or terminus.

The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to practice the invention commensurate in scope with these claims. There are many factors to be considered when determining whether there is sufficient evidence to support a determination that a disclosure does not satisfy the enablement requirement and whether any necessary experimentation is undue. These factors include, but are not limited to: quantity of experimentation necessary; amount of direction or guidance presented; presence or absence of working examples; nature of the invention; state of the prior art relative skill of those in the art; predictability or unpredictability of the art and breadth of the claims, each of which will be discussed below.

The claims are directed to a tandem fluorescent protein construct, comprising a donor fluorescent protein moiety, an acceptor fluorescent protein moiety and a linker moiety that couples the donor and acceptor moieties and wherein the donor and acceptor moieties exhibit FRET when the donor moiety is excited by radiation, characterized in that the linker moiety comprises a protease cleavage recognition site, wherein cleavage of the linker by a protease results in a change in FRET between the donor and acceptor moieties. The specification on page 8, line 12-15 appears to describe linkers as encompassing in scope those molecules that can be fluorescent in the same manner as the donor and acceptor moieties (in defining the "linker moiety" as a "radical" in the same manner as the fluorescent protein moieties). The specification

only provides guidance for the use of linkers as a non-fluorescent moiety that provides at least the appropriate degree of separation between donor and acceptor moieties. There is no guidance to use the linker in any other manner, and the effect of having an additional fluorescent moiety between the donor and acceptor would have unpredictable consequences on resonance transfer, which as taught on page 12 of the instant specification is extremely sensitive to the degree of separation between donor and acceptor. One of skill in the art would have to engage in undue experimentation to provide linkers with the properties encompassed by the claims given these factors. The claims are also directed to donor and acceptor moieties comprising SEQ ID NO:2 comprising several amino acid substitutions. Therefore the claims encompass undefined structures or multiple fluorescent moieties for which the specification is not enabled. To construct and test the many protein fragments encompassed in the claim to see the desired properties are retained would require undue experimentation.

Additionally, the specification fails to describe or provide any identifying characteristics or properties for the "other mutations" encompassed in the open claim language or provide data to demonstrate that function is retained or that the protein moieties exhibit FRET. Therefore, while it is known that many amino acid substitutions are generally possible in any given protein the positions within the protein's sequence where such amino acid substitutions can be made with a reasonable expectation of success are limited, as certain positions in the sequence are critical to the protein's structure/function relationship. For example, Heim et al. (PNAS, vol. 91, pages 12501-04, 1994) disclose that a mutated DNA was sequenced and found to contain five amino

acid substitutions, only one of which was found to be critical, Tyr66His, in the center of the chromophore. Heim et al. also disclose further site directed mutagenesis and noted that there was tolerance of the substitutions made, however, some mutants were weakly fluorescent (page 12504). The substitutions contemplated by the instant invention is greater than that proposed in the art, hence the specification should provide guidance as to what portion of the sequence is conserved and define the "other mutations" encompassed in the "comprising" language.

In addition, the specification on page 20, line 31 discloses that the optimal distance between the donor and acceptor sites is between about 1nm to about 10nm for the claimed resonance energy transfer to be useful. However, the "fluorescent protein moieties" encompass fluorescent peptide fragments of the intact fluorescent proteins, the distance between donor and acceptor may be about as short as the length of the linker. On page 20 of the specification it is stated that the length of the linker moiety is chosen to optimize both FRET and the kinetics and specificity of enzymatic cleavage. Thus, if the linker is too short, the protein moieties may sterically interfere with each other's folding or with the ability of the cleavage enzyme to attack the linker. However, the claims broadly encompass linkers that are greater than 5-50 amino acids or 1-10nm in length which is not supported by the instant specification that discloses that linker length is a critical parameter required for the tandem conjugates to work and that linker lengths beyond about 1-10nm would unpredictably result in interference with polypeptide folding, enzyme cleavage, insufficient resonance transfer, or linker cleavage specificity. Moreover, the claims recite two fluorescent protein moieties said to be linked

to one another via a linker moiety, the specification does not provide guidance as to covalent binding occurring via cyclization and oxidation of amino acids of the donor and acceptor protein moieties, or via any other methods considered to produce the "coupling" of the donor and acceptor protein moieties. No information is provided as to how the individual fluorescent moieties are to be isolated and ultimately linked to one another via any linking moiety. Thus, absent adequate guidance/direction regarding for example, the linker length, based on the breath of the claims, the undefined structures encompassed by the claims, the nature of the invention and the unpredictability of the linker as recited in the claims, a skilled artisan would not be able to practice the claimed invention commensurate in scope with the claims.

In view of the foregoing, one of skill in the art would require guidance, beyond that provided in the instant specification, in order to make the claimed tandem fluorescent protein in a manner that reasonably correlated with the scope of the claims. Without such guidance, the experimentation left to those skilled in the art is undue.

The Basis For Non-Statutory Double Patenting

6. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See *In re Goodman*, 11

F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970);and, *In re Thorington,* 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.130(b).

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

7. Claims 79-84 and 91-99 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-13 and 43-44 of U.S. Patent No. 6,803,188. Although the conflicting claims are not identical, they are not patentably distinct from each other because the claims in each are directed to tandem fluorescent protein constructs comprising a donor fluorescent moiety, an acceptor fluorescent moiety linked by a linker moiety, wherein the donor and acceptor moieties exhibit fluorescence resonance energy transfer (FRET) when said donor is excited and wherein the linker moiety has a protease cleavage recognition site. Both sets of claims recite substitutions that can occur to the donor and acceptor moieties which comprise an *Aequorea* fluorescent protein with respect to SEQ ID NO:2. Note

that the modifications contemplated in the patent are encompassed in the instant application and therefore the limitations in the instant application are considered obvious in light of the patented claims; the claims of the patent are generic to the instant claims. Therefore, the claims of the patent and the instant application claims are an obvious variation of each other.

Response to Arguments

8. The response filed has been considered, however, is not fully persuasive. Note that the rejections under 35 U.S.C. 112, first paragraph, written description and enablement remains for the reasons stated above and herein. Also note that a new ground of rejection has been instituted under 35 USC 112, first paragraphs and a rejection of Obvious-type double patenting.

Regarding the rejection under 35 U.S.C. 112 first paragraph written description, applicant state that 'any contiguous 150 amino acid residue of the fluorescent protein that has a sequence of 85% is required by the claim. Applicant also points to case law and a table in the specification (said to be on pages 21 and 24). However, no tables were found on pages 21 or 24. Table II can be found on page 22 which provides the length of the linkers, however does not rectify all the issues raised. Applicant's comments have all been considered but are not persuasive. The issue at hand is that the claims are genus claims. The claims encompass a large variable genus not

adequately described. The claims are not limited to the examples provided in the specification. Furthermore, the rejection remains because the recited 85% simply does not meet the revised written description guidelines. It is noted that claim 79 for example has specific positions to be mutated, however, the open language comprising means that other positions can also be mutated and the guidelines clearly sets forth 95% plus function. Thus the rejection remains.

Regarding the rejection under 35 U.S.C. 112, first paragraph enablement, applicant state that the specification teaches how to generate a tandem AvGFP-rp in which both fluorescent moieties contain 38 mutations with respect to the wild type protein sequence, and which still retains useful, if not improved, fluorescent activity. Applicant also states that the state of the art and the amount of experimentation are inter-related. Applicant's conclude that in view of the extensive disclosure the invention can be practiced with an amount of experimentation which would be routine in the art. This argument is not persuasive. Applicant's make the statement that fewer than 23 amino acid differences exist with respect to the wild type protein. The art sets forth that a third of single amino acid changes would completely inactivate the average protein and the more substitutions made the more probability that the protein will be inactivated. Thus, this gives the sense of what one of skill in the art can expect when a claim embraces fragments with up to 10, 20, 30, 40 or more amino acid changes and how many mutants one of skill in the art can test in such an endeavor (see Guo et al., PNAS, vol. 101, no.25, pages 9205-9210, 2004). Applicant also state for clarification purposes that the linker moiety is a linker radical that serves to join the acceptor and donor

moieties.

Contrary to applicant's statements that a relative skilled and experience artisan would not consider this to be undue experimentation, the instant application is an invitation to engage in undue experimentation absent sufficient guidance as to what residues will be affected or evidence that said sequence can tolerate the variability contemplated or information on conserved regions/domains. The issue at hand is the breath of the claims in view of the art and guidance provided in the specification. The specification defines the linker as a radical and also defines a fluorescent protein moiety as a radical, which is problematic with respect to the energy transfer desired (see page 8). The other issue raised is that the claims broadly read on any sequence that possess the mutations set forth in claim 79, as the instant specification clearly delineate whether a sequence that has 85% sequence identity will have all the characteristics desired.

The issue in this case is the breath of the claims in light of the predictability of the art as determined by the number of working examples, the skill level artisan and the guidance presented in the instant specification and the prior art of record. This make and test position is inconsistent with the decisions of *In re Fisher, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970)* where it is stated that "...scope of claims must bear a reasonable correlation to scope of enablement provided by the specification to persons of ordinary skill in the art...". Without sufficient guidance, determination of having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily and improperly extensive and undue. See *In re*

Wands, 858 F.2d at 737, 8 USPQ2d at 1404 (Fed. Cir. 1988). Thus, the rejection remains.

Conclusion

9. No claims are allowable..

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Hope A. Robinson whose telephone number is 571-272-0957. The examiner can normally be reached on Monday-Friday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Nashaat T. Nashed, Ph.D., can be reached at (571) 272-0934. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

HOPE ROBINSON
PRIMARY EXAMINER

Hope Robinson, MS

Primary Examiner